

The slide to be stained for Vimentin (VIM) comprised:

1. Endocervical adenocarcinoma, 2. Appendix, 3. Colon with metastatic malignant melanoma, 4. Seminoma, 5. Endometrioid endometrial adenocarcinoma.

Criteria for assessing a VIM staining as optimal included: A strong and distinct cytoplasmic staining of virtually all normal mesenchymal cells such as endothelial cells, fibroblasts, macrophages and lymphocytes, as well as the malignant melanoma and the endometrioid adenocarcinoma. In the seminoma, a heterogeneous, often dot-like staining should be seen. No staining should appear in the enterocytes of the appendiceal mucosa or the endocervical adenocarcinoma.



79 laboratories submitted stainings. At the assessment 60 stains were considered optimal (76 %), 14 good (18 %), 3 borderline (4 %) and 2 (3 %) poor.

The following Abs were used:

mAb clone V9 (DakoCytomation, n=35; BioGenex, n=4; Ventana, n=3; Novocastra, n=2; and Zymed, n=1)

mAb clone 3B4 (DakoCytomation, n=30; Ventana, n=3; & Novocastra, n=1)

mAb clone SP20 (NeoMarkers, n=1)

Optimal staining could be obtained with any of the 3 mAbs: 40 out of 44 (91 %) using clone V9, 19 out of 34 (56 %) using clone 3B4, and 1 out of 1 using clone SP20.

In the protocols with clone V9, optimal stainings were obtained using HIER with either Tris-EDTA/EGTA pH 9 as the heating buffer (24 out of 25 (96%)), Citrate pH 6 – 7,3 (12 out of 15 (80%)), EDTA pH 8 (1 out of 1), or TRS High pH DakoCytomation (1 out of 1), or CC1 (Ventana Benchmark) (2 out of 2).

In the protocols with clone 3B4, optimal stainings were obtained using HIER with Tris-EDTA/EGTA pH 9 as the heating buffer (16 out of 18 (89%)) or Citrate pH 6 (2 out of 6). With proteolytic pre-treatment optimal staining could be obtained in 1 out of 8 cases.

In the protocol with clone SP20 the optimal staining was obtained with HIER using Tris-EDTA/EGTA pH 9 as the heating buffer.

The most frequent causes of insufficient stainings were:

- Inappropriate epitope retrieval (excessive proteolytic pre-treatment)
- Omission of epitope retrieval

Proteolytic pre-treatment (Proteinase K) often resulted in over digestion of the tissue causing loss of cytoplasm and consequently false negative staining. This was particularly seen in the seminoma.

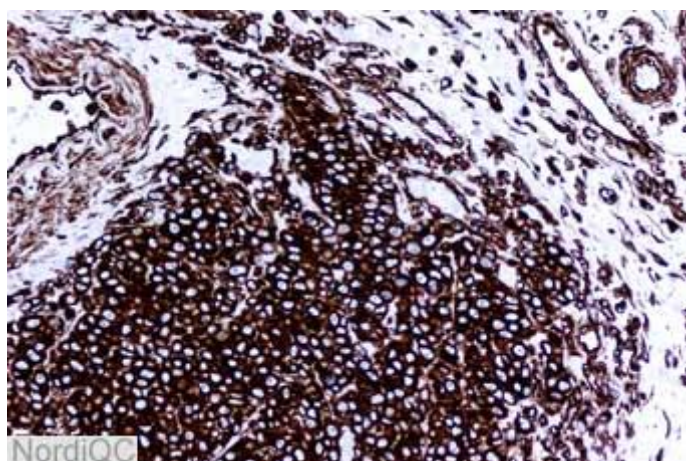


Fig. 1a
Optimal vimentin staining of the malignant melanoma infiltrating the colon. All the neoplastic cells as well as endothelial cells and lymphocytes are strongly stained.

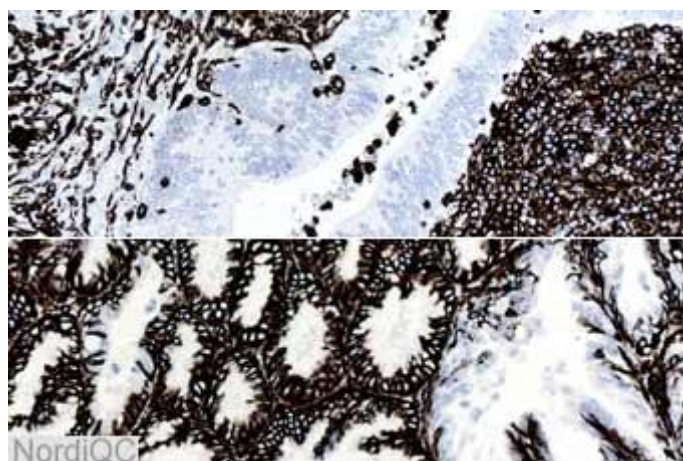


Fig. 1b
Upper part: Optimal vimentin staining of the endocervical adenocarcinoma. The neoplastic cells are negative, while lymphocytes and fibroblasts are strongly stained.
Lower part: Optimal vimentin staining of the endometrioid adenocarcinoma. The majority of the neoplastic cells show a strong cytoplasmic staining.

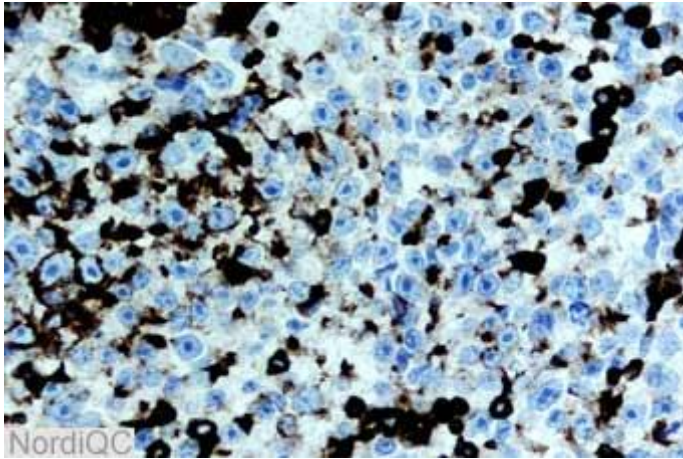


Fig. 2a
Optimal vimentin staining of the seminoma. The neoplastic cells show a heterogenous reaction. The majority of the cells display a dot-like reactivity and a few a strong cytoplasmic reaction.

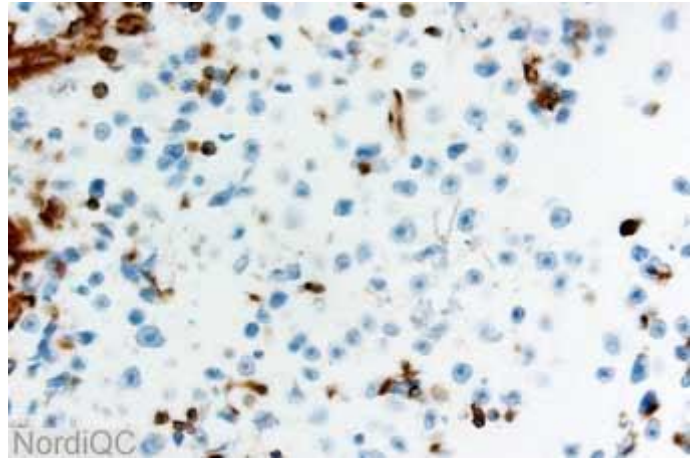


Fig. 2b
Insufficient vimentin staining of the seminoma. The neoplastic cells are false negative primarily due to excessive proteolysis. The slide looks "empty" because of digestion of the tumour cell cytoplasm.

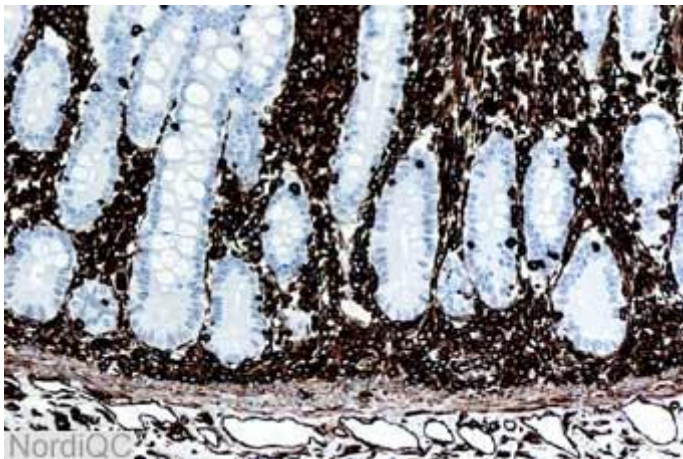


Fig. 3a
Optimal vimentin staining of the appendix. All lymphocytes and endothelial cells are strongly stained. The smooth muscle cells in the lamina propria show a moderate reaction, whereas the mucosal enterocytes are negative.

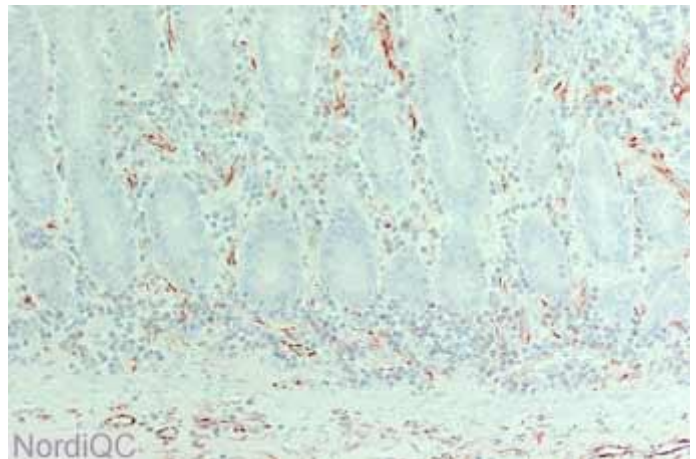


Fig. 3b
Insufficient staining of the appendix. Only endothelial cells are weakly stained, while lymphocytes, smooth muscle cells etc. are false negative. In this case the cause was omission of epitope retrieval.

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